Pretreatment

Dan Schell, Rick Elander, and Jim McMillan

Abstract

Last year we increased our understanding of feedstock compositional variability on pretreatment performance. In particular, we demonstrated that hemicellulosic sugar yields and enzymatic cellulose digestibility improve with increasing feedstock carbohydrate content. We also achieved significant progress in improving the operability and extending the solid concentration operating range of the pilot-scale, continuous pretreatment reactor from 25% up to 30% solids. This will serve us well in our continuing efforts to advance understanding of pretreatment processes, in particular, understanding the role of biomass composition and structure on pretreatment, and how process chemistry, hydrodynamics, and mass transfer are affected during high-solids concentration, dilute sulfuric-acid pretreatment. In addition, we would like to gain further insight into factors limiting enzymatic cellulose hydrolysis. Developing a higher level of insight will require us to develop tools to study how enzymatic cellulose hydrolysis correlates with the fine structure of pretreated biomass.

Recommendations for future work include 1) continue to generate and supply raw, pretreated, and enzymatically-digested materials to program subcontractors and other external stakeholders, and 2) continue fundamental research into high-solids, dilute sulfuric-acid pretreatment. The work will be conducted with corn stover as the model feedstock because we believe information generated on this material will readily translate to other lignocellulose materials, especially agricultural residues and grassy species. Specific work plans for item 2 include performing high solids pretreatment (>30%) to determine impact on hemicellulosic sugar yields and enzymatic cellulose digestibility, improving overall mass balance pretreatment by applying newly developed analytical tools for soluble protein and uronic acids, and continuing to explore the relationship between enzymatic cellulose digestibility and the physiochemical properties of pretreated biomass.

Introduction

Pretreatment of lignocellulosic biomass has been an actively researched field for several decades, and a wide variety of thermal, mechanical, and chemical pretreatment approaches (and combinations thereof) have been investigated and reported in the scientific literature (McMillan 1994). The objective of pretreatment is to hydrolyze hemicellulose and/or cellulose, which are the structural biomass carbohydrates in biomass, to produce fermentable sugars and/or enzymatically digestible cellulosic solids. In general, pretreatment can be effectively viewed as a series of reversible and irreversible endothermic reactions in which a biomass carbohydrate polymer, e.g., hemicellulose, is progressively hydrolyzed to oligomers and then monomers, which can then continue to react to form undesirable degradation products, as follows:

$$\text{Carbohydrate Polymer} \longleftrightarrow \text{Oligomeric Intermediates} \longleftrightarrow \text{Desired Monomer} \longrightarrow \text{Degradation Products}$$

The challenge is to maximize conversion of carbohydrate polymer to the desired monomer while minimizing the loss of the desired monomer to degradation products. Kinetic modeling work has repeatedly shown that yields are maximized in such a reaction scheme by operating at high temperature and short residence time.

Modern pretreatment approaches have evolved from traditional thermochemical biomass hydrolysis processes that were developed prior to World War II. These processes typically employed cooking of biomass with an acid catalyst (often hydrochloric or sulfuric acid) in a pressurized reactor to hydrolyze the cellulose fraction of biomass to glucose. In such processes, yields of glucose are typically no higher than about 60%, as the harsh conditions required for cellulose hydrolysis result in a significant fraction of the released glucose being converted to non-fermentable sugar degradation products such as 5-hydroxymethylfurfural. In addition, single stage processes designed for cellulose hydrolysis resulted in the loss of carbohydrates from the hemicelluose fraction, which is primarily derived from a pentose sugar backbone in hardwoods, herbaceous plants, and typical agricultural residues, and which is generally hydrolyzed and degraded under conditions less severe than those required for cellulose hydrolysis. Until the advent of efficient pentose utilizing microorganisms for ethanol production, the loss of carbohydrates from the hemicellulose fraction was not an important consideration, as efficient means of converting such sugars to ethanol did not exist and there were potential uses of furfural, the principal degradation product from pentose sugars, as a co-product.

The discovery of cellulase enzymes and the subsequent development of an industrial cellulase industry, coupled with the availability of efficient pentose-fermenting microorganisms, have dramatically altered the way in which the pretreatment of biomass is approached. Rather than requiring a thermochemical process to hydrolyze cellulose to glucose, the pretreatment step now needs to produce a solid substrate in which the cellulose can be efficiently digested by cellulase enzymes. It is also important that the hemicellulose-based fraction of biomass be converted at high yields to soluble pentose monomeric and/or oligomeric sugars, or minimally, be preserved as unconverted hemicellulose for subsequent enzymatic conversion, as more than one-third of the potentially available ethanol from the carbohydrates initially present in typical biomass feedstocks is hemicellulose-based. Unfortunately, these two required traits of a pretreatment process begin to illustrate the challenge of pretreatment. Mild processes that remove or alter lignin as a means of improving enzymatic cellulose digestibility do not generally release the hemicellulose sugars in a form that allows direct microbial utilization and thus require the proper enzyme activities to ultimately release monomeric pentose sugars. While some commercially available "cellulase" preparations may have specific or non-specific activity toward pretreatment-altered hemicellulose, efforts to control the cost of enzymes utilized in the bioethanol process could result in a situation where the simple fact of requiring "extra" enzymatic carbohydrate hydrolysis (i.e. both cellulose and hemicellulose) will increase the amount and/or types of protein required, potentially increasing cost. Conversely, more severe pretreatment processes that thermochemically hydrolyze all or most of the hemicellulose to soluble monomeric and/or oligomeric sugars often do so at the expense of higher temperatures, a corrosive environment that requires more expensive pretreatment reactor equipment, and loss of released hemicellulosic sugars to non-fermentable and potentially toxic sugar degradation products. Pretreatment technology evaluation and selection efforts within the Enzyme Sugar Platform (ESP) Project must consider these often-conflicting process implications.

The objective of the ESP Project is to investigate fundamental process development and integration to broadly support related Bioenergy Solicitation award winners. For instance, ESP Project researchers could develop and apply new tools and techniques that enable improved mechanistic understandings or other as-yet-to-be-identified insights about enzymatic-hydrolysis-based processes to be established. Similarly, fundamental process integration studies could be useful to identify key process interactions that govern process performance. This focus is aligned with comments received from reviewers at the ESP Project Gate 3 review. To support this effort, we performed dilute sulfuric acid pretreatment in a pilot-scale reactor to supply representatively pretreated material in large quantities for our own research efforts and generate material for external partners and other stakeholders. We also coordinate our efforts with NREL's Advance Pretreatment Task to assess the status of alternative pretreatment technologies. However, fundamental tools we develop may have broad applicability or be adaptable to the different pretreatment technologies that are being developed.

Background

Early Technology Evaluation Work

In late 2001, the ESP Project conducted a survey of pretreatment technologies to assess the status of promising pretreatment technologies that could be further investigated by this project. However, we also wanted to select one pretreatment to begin initial process development work. The initial selection effort was accomplished through a literature survey of pretreatment technologies, information obtained from and discussions with the Biomass Refining Consortium for Applied Fundamental and Innovation (CAFI) and other pretreatment researchers, and recent corn stover pretreatment results preformed in our pilot-scale reactor and by the Pretreatment Research Group at NREL. The list of pretreatment technologies evaluated is shown in Table 1.

The ESP project recommended at the Gate 3 review meeting (in January 2002) the use of a dilute sulfuric acid pretreatment process for the initial Stage 3 development efforts, since this is the only pretreatment that met all of the project's selection criteria at this time. More details of the evaluation process can be found at the provided link, http://www.ott.doe.gov/biofuels/pdfs/stage2_details.pdf . Since most of the pretreatments are under development, NREL's Advanced Pretreatment project is tracking developments and will provide feedback to the ESP Project.

Table 1. List of pretreatments investigated during ESP's pretreatment evaluation studies

Pretreatment Category	Pretreatments Undergoing Second Screen	Reactor Configuration	Information Source	Technology Developers and Providers
Base-Catalyzed	AFEX/FIBEX*	Batch/Continuous	Bruce Dale/Michigan State	Bruce Dale, MBI
	Ammonia*	Percolation	Y.Y. Lee/Auburn	Y.Y.Lee
	Lime*	Batch	Mark Holtzapple/Texas A&M	Mark Holtzapple
Non-Catalyzed	Hot Water*	Batch, Percolation	Charlie Wyman/Dartmouth, Literature	Charlie Wyman, Mike Antal/Hawaii Natural Energy Institute
		Percolation	Literature	Mike Antal, Charlie Wyman
	Hot Water-pH Neutral*	Batch	Literature, Michael Ladisch/Purdue	Michael Ladisch
Acid-Catalyzed	Nitric Acid	Batch	Scott Lynn/HFTA	Lee MacLean/HFTA
	Sulfur Dioxide	Batch or Continuous	Literature	Jack Saddler/UBC, Esteban Chornet/ University of Sherbrooke
	Sulfuric Acid*	Continuous	Literature and NREL	BC International, Iogen, NREL, TVA, Charlie Wyman, Y.Y Lee
		Batch	Literature and NREL	

Pretreatment Category	Pretreatments Undergoing Second Screen	Reactor Configuration	Information Source	Technology Developers and Providers
		Batch/Hot Wash Process	NREL	NREL
Solvent-Based	Organosolv (Clean Fractionation)	Batch	NREL	NREL
Chemical-Based	Peroxide	Percolation	Literature	
	Wet Oxidation		Ed Lehrburger/Pure Vision	Ed Lehrburger/Pure Vision

*Pretreatments being research by CAFI members

In FY2002, NREL's Advanced Pretreatment Task undertook an extensive review of pretreatment technologies. Numerous biomass pretreatment approaches have been and continue to be investigated and developed for use in bioethanol and other bio-based chemical processes. In the past, efforts to meaningfully compare and evaluate different pretreatment approaches has been hampered due to a lack of a coordinated effort to conduct experiments, perform chemical analyses, report data, and conduct process economic evaluations on a common basis across various research institutions.

This work called for the development of initial screening criteria to determine which pretreatment approaches have the potential to meet 2010 Biofuels Program targets associated with pretreatment process performance and process cost. A comprehensive set of 14 individual criteria was developed and applied to 13 unique pretreatment approaches, resulting in an overall assessment of each pretreatment approach. Since several comprehensive research projects are underway that are designed to provide the process information and results needed to rigorously evaluate the ultimate potential of various pretreatment approaches, it is premature to select or eliminate pretreatment processes that possess features that may allow them to potentially meet the advanced technology targets. However, this evaluation did indicate that three pretreatment approaches (steam autohydrolysis, solvent-based pretreatment, and biological pretreatment) do not appear likely to meet advanced technology pretreatment process performance and cost targets and are not recommended for consideration as stand-alone pretreatment approaches. Some of these approaches could find utility as a precursor or follow-up to some other pretreatment approach.

Early Pilot-Scale Pretreatment Work

Our initial work on dilute sulfuric acid pretreatment of corn stover began in late FY2000 and continued into FY2001. Pretreatments were performed in a pilot-scale, continuous 1 ton/d reactor at a reactor solids concentration of 20% (w/w) over a range of conditions encompassing residence times of 3-12 minutes, temperatures of 165-195°C, and sulfuric acid concentrations of 0.5%-1.4% (w/w) (Schell et al. 2003). We utilized a large batch of corn stover we obtained from Biomass AgriProducts (BMAP) located in Harlan, IA and later specified as Lot 1. Xylan conversion yield and carbon mass balance data were collected at each run condition. Performance results were used to estimate kinetic model parameters assuming biphasic hemicellulose hydrolysis and a hydrolysis mechanism incorporating formation of intermediate xylo-oligomers. In addition, some of the pretreated solids were tested in a simultaneous saccharification and fermentation (SSF) process to measure the reactivity of their cellulose component to enzymatic digestion by cellulase enzymes. Monomeric xylose yields of 69%-71% and total xylose yields (monomers and oligomers) of 70%-77% were achieved with performance level depending upon pretreatment severity. Kinetic model predictions for monomeric and total (monomers plus oligomers) xylose yields as a function of temperature and residence time are illustrated in Figure 1. Cellulose conversion yields during simultaneous saccharification and fermentation of 80%-87% were obtained for some of the most digestible pretreated solids.

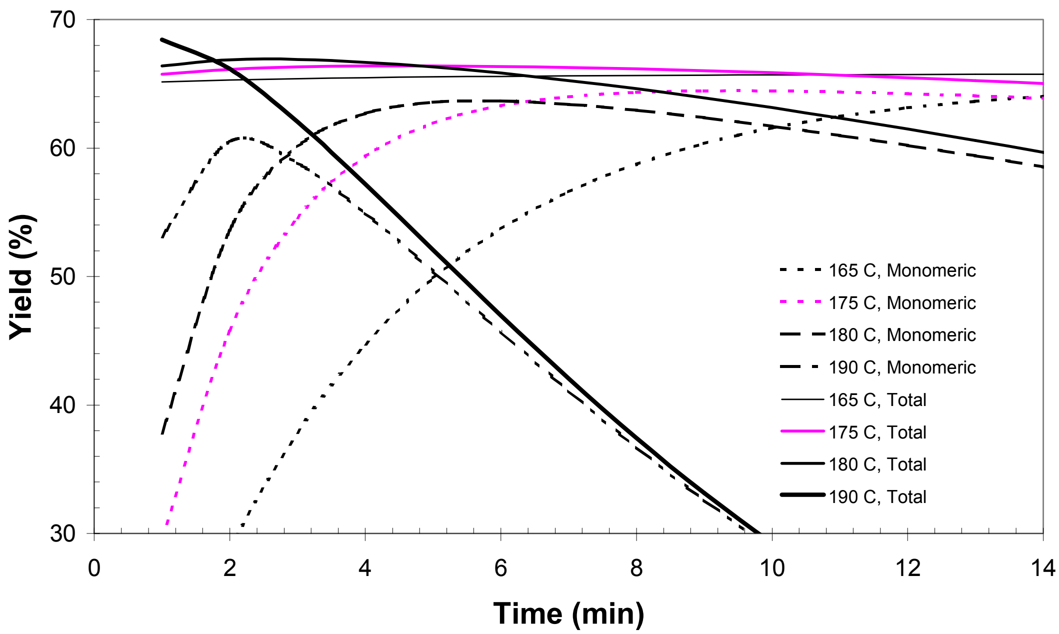

Figure 1. Xylose yields predicted by the kinetic model for Lot 1 corn stover at 20% solids concentration

Recent Pretreatment Results

Effect of Corn Stover Compositional Variability on Xylose Yields and Enzymatic Digestibility

Our initial objective was to characterize hemicellulosic sugar yields and enzymatic digestibility of pretreated of corn stover as a function of operating conditions. Although initial efforts led to the development of a kinetic model for one lot of corn stover as reported above it soon became apparent from investigations of other lots of corn stover with different compositions that a robust kinetic model could not be developed because performance was being dramatically influenced by compositional variability. Therefore, we investigated the effect of corn stover compositional variability on pretreatment and enzymatic cellulose hydrolysis performance.

Dilute sulfuric acid pretreatments were performed in the pilot-scale pretreatment reactor using three different lots of corn stover obtained from BMAP. Pretreatment performance was assessed from data on xylan hydrolysis and enzymatic cellulose digestibility at different pretreatment conditions and by replicating a few pretreatment conditions many times in order to apply statistical techniques to quantify performance differences. Table 2 list pretreatment conditions and replicated samples and also identifies samples tested for enzymatic digestibility, since this test was not performed on every sample. Enzymatic digestibility was determined as ethanol yield (after 7 days) from cellulose during SSF at 32°C, 15 FPU/g cellulose enzyme loading using a fermentative yeast. When appropriate (i.e., sufficient samples available), Student-t testing was used to determine if these differences were significant. Selected samples from different corn stove lots pretreated at the same conditions but displaying dramatic performance differences were subjected to additional testing. These tests included NMR spectroscopy, electron microscopy, and enzyme adsorption and saccharification.

Table 2. List of samples used in the performance comparison study and corresponding pretreatment conditions

Pretreatment Conditions[a]			Feedstock Lot		
Temperature (°C)	Acid Concentration (%, w/w)	Time (min)	1	2	3
165	1.4	8	P001220 #1 P001220 #4* P010116 #5 P010129 #1* P010129 #2 P010517 #3*	P011219 #1* P020820 #1* P020820 #3*	P020311 #1* P020311 #6* P020603 #1* P020605 #4*
175	0.95	8	P001116 #1*[b] P001128 #4*	P011219 #5* P020820 #2*	P020311 #3*[b] P020425 #1* P020605 #3*
190	1.2	c	P001207 #5*	P011211 #4* P020820 #4*	P020311 #4* P020425 #3* P020605 #1* P020605 #2

[a]all pretreatments performed at 20% solids concentration
[b]ultimately discarded from analysis due to poor mass balance closure
[c]reactor operated in a flow-through mode that achieves a constant residence time estimated at 0.75-1.25 min
*cellulose digestibility measured by SSF testing

The average compositions of the raw corn stover lots used in this work are shown in Table 3. Except as noted, most of the values reported in this table are based on NIR measurements. Since the current NIR model does not accurately predict levels of the minor carbohydrates (galactan, arabinan, and mannan), acetyl and ash, these components will not be further discussed. Total cellulose plus xylan content is highest in Lot 2 corn stover and decreases in order from Lot 2 to Lot 3 and finally to Lot 1 and these differences are significant at a 95% confidence level.

Table 3. Average composition (% w/w, dry basis) of corn stover used in this study as measured by NIR (except as noted). Standard deviations are shown in parenthesis.

Corn stover sample	Cell.	Xyl.	Gal.	Ara.	Man.	Lignin	Ash	TGX
Lot 1 (5 samples)[a]	37.1 (0.4)	19.2 (0.5)	1.6 (0.1)	2.5 (0.3)	1.3 (0.5)	20.7 (0.1)	5.2 (1.2)	57.0
Lot 2 (13 samples)[b]	38.7 (0.9)	23.2 (0.6)	1.8 (0.4)	1.9 (0.87)	1.2 (0.4)	21.3 (1.9)	2.4 (1.5)	61.9
Lot 3 (17 samples)	36.6 (0.6)	21.6 (0.5)	1.9 (0.1)	1.7 (0.4)	1.0 (0.2)	19.4 (1.0)	4.0 (0.5)	58.2

Cell. – Cellulose, Xyl. – Xylose, Gal. – Galactose, Ara. – Arabinose, Man. – Mannose, TGX – Total Cellulose+Xylan

[a]all samples measured by wet chemistry

[b]only three samples measured by wet chemistry

This work was motivated by results shown in Figure 2, which shows total xylose yields as a function of the combined severity factor for the different corn stover lots. The combined severity factor is a rough indication of pretreatment severity and is useful for comparing large data sets. The figure shows that total xylose yield results from Lot 2 are clearly better than results for Lot 1. This suggests that stover compositional differences significantly influence pretreatment performance.

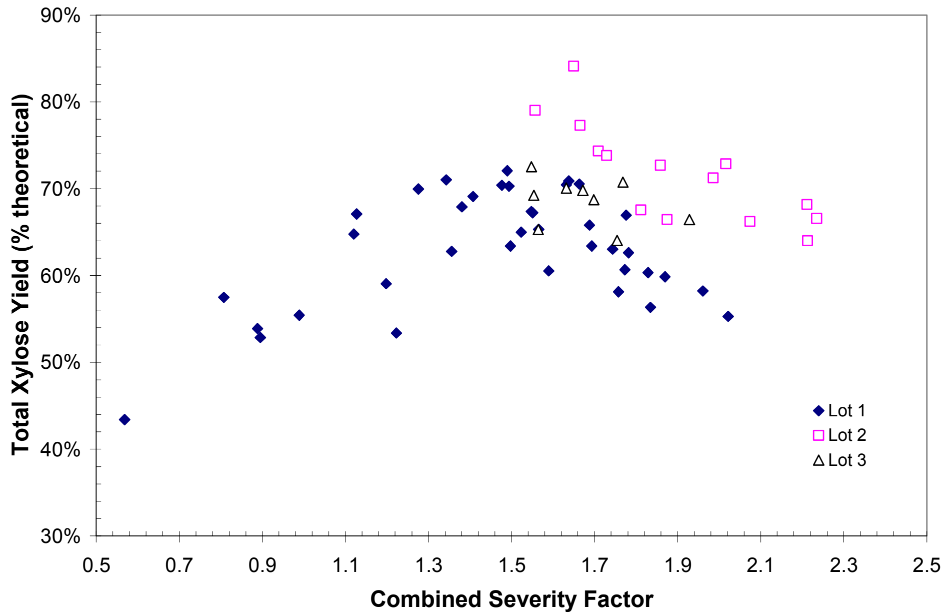

Figure 2. Total xylose yield as a function of the combined severity factor

This hypothesis was investigated in more detail by repeatedly testing the different corn stover lots at the same pretreatment conditions using the samples listed in Table 2. Average ethanol (from SSF testing), monomeric, and total xylose yields along with one standard deviation error bars for a 165°C, 1.4% (w/w) acid, and 8 min pretreatment are shown in Figure 3. Examination of these results suggest that the lots can be ranked in order of increasing performance for both cellulose conversion and pretreatment performance from Lot 1 (lowest) to Lot 3 to Lot 2 (highest). This was verified at a greater than 95% confidence level for xylose hydrolysis differences between Lot 2 and the other two lots. Less significant (>80%) confidence level differences in xylose yields are noted between Lots 1 and 3. Ethanol yield differences were less noticeable, although, the difference in ethanol yield between Lot 1 and 2 is significant at an 80% confidence level. The same trends were observed for the other two pretreatment conditions listed in Table 2.

Other tests of the similarly pretreated samples did not reveal additional significant differences between the samples. That is, NMR and SEM results were inconclusive and enzymatic digestibility, saccharification, and enzyme (protein) adsorption performed as expected with Lot 2 pretreated material performing better than similarly pretreated Lot 1 material and the poorly pretreated material.

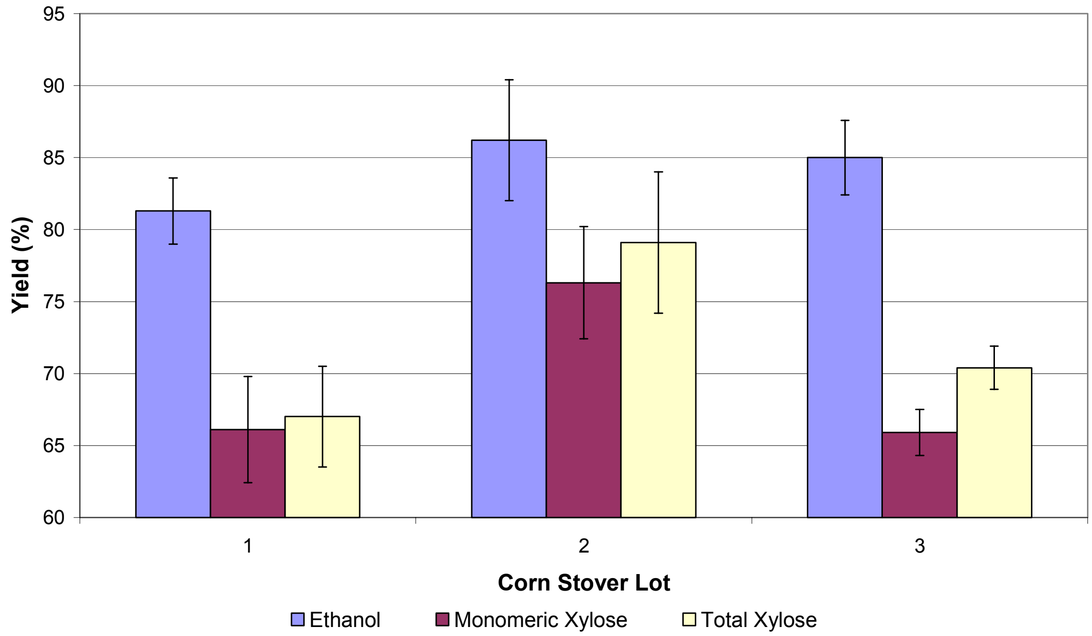

Figure 3. Average ethanol production by SSF, monomeric xylose, and total xylose yields for corn stover pretreated at 165°C, 1.4% (w/w), and 8 min along with one standard deviation error bars.

The results of this study demonstrate that significant performance differences exist between pretreated samples generated from different corn stover lots, which differed significantly in composition. Both hemicellulosic sugar yields from pretreatment (up to 18% higher yields) and enzymatic hydrolysis of the residual cellulose (up to 6% higher yields) was higher from samples pretreated from Lot 2 material when compared to the other two lots. The data suggest that the higher carbohydrate content of Lot 2 material can be correlated with better performance, although we cannot conclude that carbohydrate content is the only factor responsible for the better performance. Nevertheless, higher carbohydrate feedstocks will significantly improve process economics due to their greater ethanol production potential.

Effect of Solids Concentration on Pretreatment Performance

The pretreatment reactor solids concentration is a key factor significantly effecting process economics for a dilute sulfuric acid pretreatment/enzymatic hydrolysis-based process. The impact is shown in Figure 4 along with progress we have made in the last two years with increasing the operating solids concentration in our pilot-scale pretreatment reactor. However, the assumption made in evaluating the process economics is that operating at higher solids loading does not negatively affect xylan conversion yields or cellulose enzymatic conversion, which is unproven. We have just began work at higher pretreatment reactor solids loading (≥ 30%) and currently only have only one data point at a 30% solids loading that we can compared with a similar pretreatment at 20% solid loading as shown in Table 4.

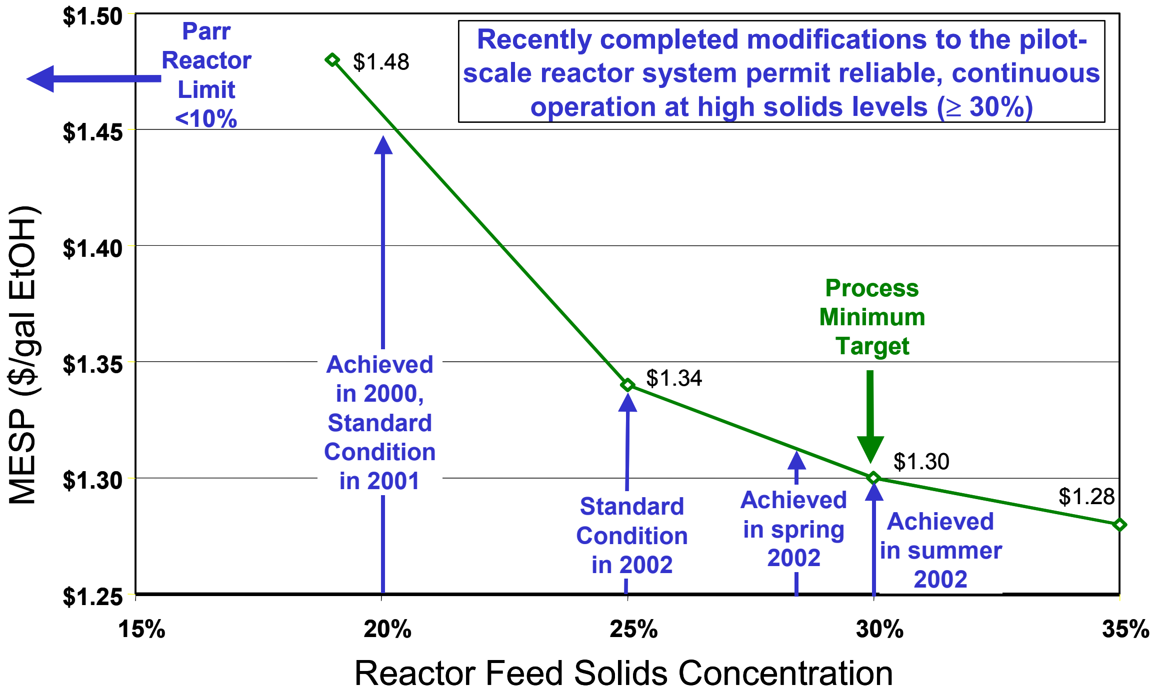

Figure 4. Cost impact of pretreatment reactor solids loading

Table 4. Representative pretreatment performance results at two different reactor solids loadings

Pretreatment Solids Concentration (wt %)	Monomeric Xylose Yield (%)	Total[a] Xylose Yield (%)	Cellulose Conversion[b] (%)	Xylose Mass Balance Closure[c] (%)	Total Sugar Concentration[d] (g/L)
20	78	85	93	104	94
30	75	78	95	89	143

[a]total of monomeric and oligomeric sugar
[b]cellulose digestibility (conversion) measured by SSF testing
[c]mass of xylan derived components (monomeric and oligomeric xylose, unreacted xylan, and furfural; corrected for hydrolysis) in the outlet streams divided by mass of xylan in
[d]total (monomeric and oligomeric) of glucose, xylose, arabinose, galactose, and mannose in the pretreated liquor stream

The two pretreatment were performed at 190°C, an estimated 1 min residence time, and at the same acid loading (0.045 g acid/g dry biomass). One word of caution, it is not known if scale up based on acid loading in fact produces "similar" pretreatment conditions. The same acid loading at higher solid loading produces a much higher liquid phase acid concentration. One could also envision scale up based on maintaining the same acid concentration. However, our limited experience and one result in the literature addressing this issue (Horwath el al. 1983) suggests that acid loading is the better factor to use. But we are not claiming that pretreatment conditions compared in Table 4 are the same.

The table shows that xylose yields at 20% are greater than at 30% as might be expected from kinetic considerations. That is, higher sugar concentrations produced by higher solids pretreatment should lead to higher rates of sugar degradation. However, xylose mass balance closure is suspiciously low for the 30% data, which suggest that these results maybe somewhat under estimated. Cellulose conversions at the two conditions are similar and interestedly somewhat higher at 30%, but not significantly. The last column in the table illustrates the significantly higher sugar concentrations that can be achieved at higher solids loadings and produce the subsequent reduction in price because higher sugar concentration translates into lower operating and capital cost. Again, it is worth reiterating that there is no certainty that pretreatment conditions were similar as discussed above and future work should investigate this issue.

Predicting Enzymatic Digestibility

Using the pretreatment results obtained to date, various factors were examined for their ability to correlate with enzymatic digestibility. All of the factor could be readily available outputs from kinetic models for dilute acid hydrolysis of hemicellulose and cellulose and thus could be used to predict cellulose enzymatic digestibility. Given our previous results discussed above one questions that needs answering is—Can these predictions be made independent of corn stover composition or is composition another factor along with solids concentration that need to be included in kinetic models?—both which have not been included in models to date. Although we cannot answer this question yet, Figure 5 shows some factors that appear to correlate with cellulose digestibility.

Cellulose digestibility, defined as the ethanol yield from cellulose after 7 days during SSF, is plotted as a function of residual xylan in the pretreated solids, relative xylan removal, absolute xylan removal, and xylan to glucan ratio (Figure 5). Previous work by Grohmann et al. (1986) (http://www.afdc.doe.gov/pdfs/672.pdf) has shown that xylan removal correlates well with cellulose digestibility. Values from each corn stover lot are plotted as different symbols to facilitate evaluation of feedstock differences. Although other factors were examined (e.g., residual cellulose concentration, total mass removed, etc.), no correlation was found. Clearly, as shown by the grouping together of similar symbols, these results are dependent upon feedstock lot, which is not unexpected based on the results previously presented. A tighter correlation would be desirable, but the scatter is probably due to measurement error or perhaps because the factor is not highly correlation with digestibility. Although, it is difficult to access which of these influences is more important. A closer examination of these results suggests that absolute xylan removal provides a slightly better correlation.

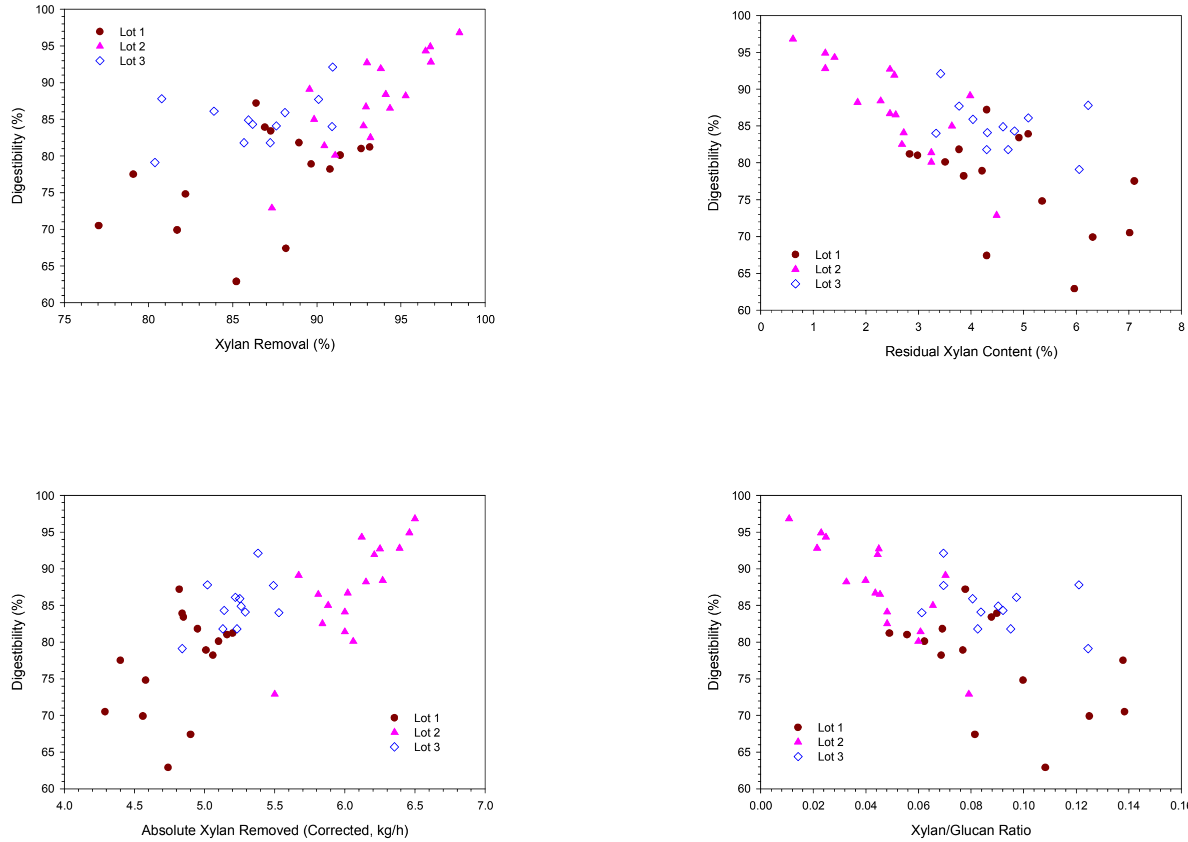

Figure 5. Correlation of cellulose digestibility with several properties of pretreated corn stover

Supplying Process Material to External Stakeholders

Although not directly related to the pretreatment effort, the ESP Project supplies raw and pretreated corn stover and/or enzymatically-digested process residue to external stakeholders for the purpose of furthering ESP and programmatic goals. From Jan.02 through Jan. 03 the project has supplied the material listed in Table 5. In the coming year, we expect to continue to supply materials to program subcontractors and other external stakeholders.

Table 5. List of material supplied from Jan. 02 through Jan. 03 to program subcontractors and other stakeholders

Material Supplied	Amount Supplied	Number of Contacts Supplied
Raw corn stover	Over 5 tons	4
Pretreated corn stover solids	30 kg (dry basis)	3
Pretreated corn stover liquor	193 L	5
Enzymatically-digested residue	67 kg (dry basis)	8

Recommendations and Future Work

The Biomass Research and Development Technical Advisory Committee has produced a roadmap document stating, "Improvements are needed to improve physical and chemical pretreatment of biomass feedstock prior to fermentation" (http://www.bioproducts-bioenergy.gov/pdfs/FinalBiomassRoadmap.pdf). Consistent with this message, DOE's Office of the Biomass Program Multiyear Plan 2003 to 2010 has identified several key technical barriers or knowledge gaps to the Biomass to Sugar Biorefinery that are particularly relevant to ESP's mission. These objectives are to advance understanding of 1) the impact of biomass composition and structure on pretreatment, 2) the cost of pretreatment options (alternative pretreatments, materials of construction, reactors), and 3) the low reactivity of current commercial enzymes. Also relevant is understanding pretreatment process chemistry, hydrodynamics, and mass transfer during high solids operation.

Complementary to understanding the role of biomass composition and structure on pretreatment is understanding the role of the complex three-dimensional structure of pretreated biomass on enzymatic cellulose hydrolysis to gain further insights into limiting factors. This could further a universal understanding of the affect of different pretreatment technologies on cellulose hydrolysis. Most work to data has focused on correlating performance with the average composition of pretreated material (see information presented earlier or Chang and Holtzapple 2000). We believe developing a higher level of insight will require us to develop tools and to study how performance correlates with the fine structure of pretreated biomass. To this end, hypotheses concerning the accessible volume/surface and other surface characteristics should be developed and tested using appropriate tools and measurements. In addition, modeling work occurring in the Advanced Pretreatment Task may provide additional information and hypothesis to test.

Recommendations for continuing and future work in the pretreatment area are:

• Continue to generate and/or supply materials (raw, pretreated, and enzymatically-digested lignocellulose biomass) to external stakeholders working to advance Biomass Program goals to support on-going process integration efforts

• Continue fundamental research in high-solids pretreatment focusing on work that advances core knowledge and also leads to reductions in the cost and risk of biomass conversion technology. This goal is consistent with the input received from the Gate 3 reviewers last year. Specifically, we propose to focus our work in the following areas, while being cognizant of and coordinating with work occurring in the Advanced Pretreatment Task that impacts our direction and goals.

- Investigate high solids pretreatment (≥ 30%) via dilute sulfuric acid hydrolysis and determine the impact on hemicellulosic sugar yields and enzymatic cellulose digestibility. As previously discussed, the pretreatment reactor solid loading has a large impact on process economics. This work will be conducted in the PDU's continuous pilot-scale pretreatment reactor and will generate new and rigorous data for process modeling. Data generated from this effort will also help develop new hydrolysis kinetic models that incorporate the effect of solid loading and attempt to predict enzymatic cellulose digestibility. The development of new kinetic models is a goal of the Advanced Pretreatment Task but will be partially supported by this effort.

- Work to improve overall mass balance closure across pretreatment by applying newly developed analytical measurements for soluble protein and uronic acids. This is important for process modeling because it contributes information on component flows that helps their impact on process economics be better understood.

- Continue to explore the relationship between enzymatic digestibility and the structure and properties of the pretreated material. This effort will seek to use tools such as porosity or pore volume measurements and other surface characterization tools such as atomic force microscopy to further elucidate the factors affecting enzymatic cellulose digestibility. This work will be closely coordinated with the Advanced Pretreatment Task.

- The work defined above will initially be conducted with a single lot/batch of corn stover to minimize variations due to compositional differences in feedstock. However, once baseline performance information is established, lot/batches of different corn stover varieties will be used in an effort to understand how compositional factors affect performance. We believe this work will facilitate industry efforts to commercialize this technology.

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